

### **REMARKS**

Claims 15-25 are cancelled as drawn to non-elected inventions. Applicants reserve the rights to file one or more continuation applications directed to the non-elected inventions.

Claims 1, 12, and 29 are amended to recite that the proteorhodopsin is membrane-free. Support for the amendment can be found at page 11, lines 12-13. The amendments are made to clarify the meaning of the claims.

Claim 10 is amended to correct the antecedent basis.

Claim 26 is amended to recite that directing light onto a selected portion of a material containing immobilized proteorhodopsin. Claim 28 is amended to recite directing light onto selected locations and selected layers of a three-dimensional optical information storage material. The amendments are to clarify the meaning of the claims as suggested by the Examiner.

No new matter is added in any of the amendments. The amendments are made to overcome the final rejection. The amendments merely clarify the meaning of the claims and do not raise a new issue or require a new search. The Examiner is requested to enter the amendments and re-consider the application.

### **102(a) Rejections**

9. Claims 11-14, 26 and 28 are rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Dioumaev et al. "Proton Transfers in the photochemical reaction cycles of proteorhodopsin", Biochem., Vol. 41(17) pp. 5348-5358 (4/2002). The rejection is traversed because Dioumaev et al. do not teach each and every element as set forth in Claims 11-14, 26 and 28, either expressly or inherently.

Dioumaev et al. only disclose the measurements of photocycle kinetics of proteorhodopsin (PR) **using PR membranes encased in polyacrylamide gels or using membrane suspensions.**

Claim 11 is directed to a fraud-proof material comprising at least two solid materials each containing immobilized proteorhodopsin having different maximum absorption wavelengths. Dioumaev et al. do not teach the claimed element of two

different proteorhodopsins encased in polyacrylamide gels. Therefore, Claim 11 is not anticipated by Dioumaev et al.

Claim 12 is directed to an optical information carrier comprising a solid material having immobilized proteorhodopsin, wherein said proteorhodopsin is membrane-free and in a monomer or an oligomer form. Dioumaev et al. did not purify PR to a monomer or an oligomer form, and only used the membrane suspension in which PR was not membrane-free and was not in a monomer or an oligomer form. Therefore, Claim 12 and its dependent Claims 13 and 14 are not anticipated by Dioumaev et al.

Regarding to Claims 26 and 28, Applicants do not agree with the rejection. However, to accelerate the allowance of the claims, Applicants have amended the claims as suggested by the Examiner.

Therefore, the 102(a) rejection of Claims 11-14, 26 and 28 over Dioumaev et al. should be withdrawn.

10. Claims 1-4, 8-14, 26, 28 and 29 are rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Friedrich et al. “Proteorhodopsin is a light driven proton pump with variable vectorality”, J. Mol. Biol., Vol 321(5) pp 821-838 (2002).

The cited claims are not anticipated by Friedrich et al. because (a) Friedrich et al. do not teach a solid material having immobilized PR, and (b) Friedrich et al. do not teach membrane-free, detergent solubilized PR. “By immobilization,” the PR molecules are fixed and do not diffuse or diffuse very slowly within the solid material, such that the optical signal is not lost by diffusion of the PR molecules. (see Application at page 6, lines 26-28)

There is no dispute that Friedrich et al. generated all of the spectroscopic data using PR reconstituted in phospholipid membrane vesicles. Applicants respectfully submit that the Examiner is incorrect in classifying dioeolylphospholipids as detergents that can solubilize PR.

At page 834, in the section of “Reconstitution of Proteorhodopsin”, Friedrich et al. describe the procedures that PR was reconstituted into dioeolylphospholipids. The PR-containing solution was added with detergent-adsorbing beads to adsorb the detergent. The beads (containing the adsorbed detergent) were removed and PR was

washed before reconstitution into dioeolylphospholipids. Dioeolylphospholipids are the primary ingredients that make the bilayer membrane vesicles; dioeolylphospholipids themselves do not solubilize PR from the membrane vesicle. This procedure resulted in the formation of large bilayer membrane vesicles containing PR, which are likely to cause light scattering. Therefore, Friedrich et al. do not teach an optical information carrier comprising detergent-solubilized and membrane-free PR (Claims 1-4, 8-10, and 29), or membrane-free PR in a monomer or an oligomer form (Claims 12-14).

As to Claim 11, Friedrich et al. do not teach the claimed elements of two different proteorhodopsins or immobilized PR in a fraud-proof material.

As to Claims 26 and 28, Applicants have amended the claims as suggested by the Examiner.

Therefore, the 102(a) rejection of Claims 1-4, 8-14, 26, 28 and 29 over Friedrich et al. should be withdrawn.

### **103(a) Rejections**

11. Claims 1-14 and 26-30 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Friedrich et al., in view of Hampp et al. '279 and Krebs et al., "Detection of fast light activated H<sup>+</sup> release and M intermediate formation from proteorhodopsin", BMC Physiology, Vol. 2 pp 5-12 (2002).

As discuss above, Friedrich et al. do not teach or suggest (a) a solid material having immobilized PR, and (b) membrane-free, detergent solubilized PR.

Hampp et al. use native purple membrane patches, which are micrometer sized patches containing a 2D crystal of lipids and bacteriorhodopsin (BR) proteins. Hampp et al. do not teach or suggest PR, let alone membrane-free, detergent solubilized PR.

Hampp et al. do not even teach or suggest membrane-free, detergent solubilized BR.

There are many differences between the present invention and Krebs. The most important difference is that Krebs et al. describe a basic research that examines the physical properties of PR in a solution phase, not in an immobilized format. Krebs et al. did flash photolysis with reconstituted PR in a solution phase. Krebs et al. do not teach or suggest a solid material having immobilized PR, wherein the PR is membrane-free and detergent solubilized.

Krebs extract PR from membrane with a detergent  $\beta$ -octyl-D-glucoside. The column-purified PR was reconstituted into mixed micelles containing 1,2-diheptanoyl-SN-glycero-3-phosphocholine (DHPC). DHPC is a phospholipid that can form micelles. DHPC is not a detergent that can solubilize PR from membrane. This is clear to a skilled person, and is also clearly indicated in Krebs et al., who describe that a PR sample was reconstituted into mixed micelles containing DHPC by adding DHPC and then removing most of the detergent ( $\beta$ -octyl-D-glucoside) on Sephadex G-25 column equilibrated with DHPC (see pages 7-8). The detergent ( $\beta$ -octyl-D-glucoside) was removed before micelles could be formed.

At page 6, last paragraph, Krebs et al. state that “The requirement for pR to be in lipid to show fast  $H^+$  release and M formation stems either from a protein/lipid interaction needed to establish a stable, active tertiary structure, or from the need for the phosphate group in DHPC to act as a proton release group.” In the second paragraph of Conclusion at page 7, Krebs et al state “The necessity of reconstituting pR with some lipid before it is capable of photocycling shows that the presence of lipids facilitates pR in assuming its fully active structure.” Krebs et al state that pR needs to be reconstituted with lipids before being capable of photocycling/M state formation, thus Krebs et al, teach away from the present invention of an optical information carrier comprising detergent-solubilized, membrane-free PR. On the contrary, in the present application, Applicants have provided a working example of optical data storage using proteorhodopsin-PVA film, where the PR is detergent-solubilized and membrane-free. (See application, Example 9).

In the Office Action at page 9, the Examiner states that the PR sample of Krebs et al are stable for several months. This is incorrect. The Krebs paper (at the beginning of page 3) states that PR stored for a few weeks in octylglucoside solution at 4°C has changed mobility on an SDS-PAGE gel, which indicates the instability of their PR sample during storage in the octylglucoside solution.

The membrane-free detergent-solubilized PR or the membrane-free monomer/oligomer form of PR has unexpected advantages over the membrane fragments-containing PR, or phospholipid vesicle-containing PR (reconstituted PR) in

that the former does not cause light scattering, thus providing a good signal-to-noise ratio (see Application at page 3, lines 25-29).

Therefore, Friedrich et al., Hampp et al., and Krebs et al., alone or in combination, do not teach or suggest an optical information carrier comprising a solid material comprising immobilized, detergent-solubilized and membrane-free PR (Claims 10, and 29-30), or membrane-free PR in a monomer or an oligomer form (Claims 12-14).

As to Claim 11, Friedrich et al., Hampp et al., and Krebs et al., alone or in combination, do not teach or suggest the claimed element of two different immobilized proteorhodopsins in a fraud-proof material. By mixing two proteorhodopsin variants with different spectral properties, it is possible to have visually different color shifts depending on the wavelength of light used to expose the material containing the proteorhodopsins. This is based on using exposure light that selectively converts one of the proteorhodopsin variants from the basal state to the M state. The other proteorhodopsin variants will predominantly remain in the basal state. No prior art has described using a mixture of two different proteorhodopsins, so Claim 11 is novel and non-obvious.

As to Claims 26 and 28, Applicants have amended the claims as suggested by the Examiner.

Therefore, the 103(a) rejection of Claims 1-14 and 26-30 over Friedrich et al., Hampp et al. and Krebs et al. should be withdrawn.

12. Claims 1-14 and 26-30 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Friedrich et al., in view of Hampp et al. '279 and Krebs et al., further in view of Wu et al. "Bacteriorhodopsin encapsulated in transparent solgel glass: A new biomaterial", Chem. Mater., Vol. 5 pp. 115-120 (1993).

Wu disclose BR encapsulated in sol-gel glass. Wu et al. do not mention proteorhodopsin. BR and PR have low sequence similarity, thus, it is not obvious that they have similar properties in optical applications.

**The disadvantages of BR** are described in the application at page 1, lines 19-27: "One of the problems with the BR-based films is that BR forms 0.2-1  $\mu\text{m}$  sized protein-lipid patches. If BR is extracted from these patches to form a monomeric protein, it

becomes unstable and is inactivated in a few days. The problem with using these BR patches in optical films is that the patches are approximately the same size as the wavelength of the light used to interface the film. This results in significant light scattering during read and write cycles, thereby increasing noise and degrading the performance of the film. Additionally, the BR patches tend to stick to each other, which result in uneven distribution of the BR protein in the film, and further degrade the performance of BR-based optical films.”

**The advantages of PR** over BR are described in the application at pages 6 and 7. “One advantage of using proteorhodopsin as an optical information carrier is that proteorhodopsin can be functionally expressed in *E. coli* to produce a large quantity (grams or kilograms) of protein economically and efficiently.” “As an optical data storage material, it is desirable to immobilize membrane-free, detergent-solubilized proteorhodopsin to avoid light scattering. Detergent-solubilized proteorhodopsin is usually in the form of a monomer, and sometimes in the form of an oligomer (dimer, trimer, tetramer, pentamer, or hexamer). Different from bacteriorhodopsin, proteorhodopsin protein is stable in its monomeric or oligomeric state for at least one month at room temperature, or one year at 4°C.”

Therefore, the 103(a) rejection of Claims 1-14 and 26-30 over Friedrich et al., Hampp et al., Krebs et al., and Wu et al. should be withdrawn.

13. Claims 1-14 and 26-30 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hampp et al. '279, in view of Krebs et al.

Hampp et al. do not teach or suggest PR, or membrane-free, detergent solubilized BR.

Krebs et al. did flash photolysis with reconstituted PR in a solution phase. Krebs et al. do not teach or suggest a solid material having immobilized PR, wherein the PR is membrane-free and detergent solubilized. The reconstituted PR (embedded in mixed micelles) is likely to cause a problem of light scattering.

The Examiner is incorrect in stating DHPC of Krebs et al. is similar to the dodecyl- $\beta$ -maltoside of the applicant. DHPC is a phospholipid that can form micelles or membrane vesicles. DHPC is not a detergent that can solubilize PR from membrane.

Therefore, the 103(a) rejection of Claims 1-14 and 26-30 over Hampp et al. in view of Krebs et al. should be withdrawn.

14. Claims 1-14 and 26-30 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hampp et al. '279, in view of Krebs et al., further in view of Wu et al.

For the same reasons stated above in Sections 12 and 13, Claims 1-14 and 26-30 are not obvious over Hampp et al., Krebs et al., and Wu et al.

### **Summary of the Non-Obviousness of the Present Invention**

None of the cited prior art have taught or suggested immobilizing detergent-solubilized, membrane-free PR, or the optical application of detergent-solubilized, membrane-free PR. Therefore, the combination of prior art does not produce the claimed invention.

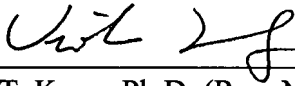
It is well known to biochemists skilled in the art working with membrane proteins that the chemical environment of a membrane protein is very different when it is incorporated into a lipid bilayer or when it is kept in solution by detergents. In most cases examined, the biochemical properties (e.g. rate constants and binding affinities) of membrane proteins are different when examined in native membranes and when detergent solubilized. Thus, it is not obvious that detergent solubilized, membrane-free proteorhodopsin will have the same properties as proteorhodopsin in membrane preparations (see Krebs et al). It is also not obvious that detergent solubilized proteorhodopsins can be immobilized into a solid matrix and be stable for a long time. The bacteriorhodopsin used by Hampp and Wu is incorporated into native archaeobacterial lipids, a very different chemical environment. Finally, proteorhodopsins show only very low sequence similarity to bacteriorhodopsin, so it is not obvious that they will have similar properties in optical applications. As an example, sensory rhodopsins and visual rhodopsins also go through photocycles when illuminated with light of suitable wavelengths. However, these proteins cannot be used in optical applications.

**CONCLUSION**

Applicant believes that the application is in good and proper condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 798-3570.

Respectfully submitted,

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